UNIVERSITY OF THESSALY

SCHOOL OF MEDICINE

LABORATORY OF BIOMATHEMATICS

POSTGRADUATE PROGRAMME (MSc):

"RESEARCH METHODOLOGY IN BIOMEDICINE, BIOSTATISTICS AND CLINICAL BIOINFORMATICS"

A PHASE 3, PLACEBO CONTROLLED, DOUBLE-BLIND, RANDOMIZED STUDY FOR ASSESSING THE EFFICACY AND SAFETY OF CORTICOSTEROIDS AND MAGNESIUM SUPPLEMENT IN PATIENTS WITH SUDDEN SENSORINEURAL HEARING LOSS

NIKOLETA BATZIOU

MASTER THESIS

SUPERVISOR:

PROF. IOANNIS STEFANIDIS

LARISSA, SEPTEMBER 2016

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EVALUATION COMMITTEE MEMBERS:

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LARISSA, SEPTEMBER 2016

PREFACE

In the context of the multi-complexity and intense research process that modern medicine is characterized by, the properly designed clinical trial protocols could enhance the role of the investigator and contribute in the production of reliable research outcomes as well as to the rational use of resources allocated to medical research.

Worldwide, a high number of people of various age groups are affected from sudden idiopathic sensorineural hearing loss. After the onset of such a disease, a negative impact on communication, relationships, and generally the quality of life of patients and their environment is hard to be avoided. There are several types of hearing loss due to a various etiology. Based on relevant literature, as well as on clinical practice, corticosteroids are considered as the best treatment by many authors; however, there is no gold standard for treating this disease because of its wide etiology. Furthermore, corticosteroids cannot be administered to patients with specific health problems and may cause specific side effects. These facts lead to the need for a treatment with less limitations and side effects in the global scientific research.

In the frame of this thesis, a protocol for a randomized clinical trial is specially designed which could be useful in investigating the effect of magnesium in patients with sudden idiopathic sensorineural hearing loss.

The thesis was elaborated in the Laboratory of Biomathematics, of the University of Thessaly School of Medicine. It is structured in 10 chapters including all the ideas, criteria and rules related to the rational design and development of a randomized clinical trial protocol.

I am indebted to express my gratitude to my supervisor Professor Ioannis Stefanidis for his useful comments, remarks and engagement through the learning process of this master thesis. Furthermore, I would like to thank Professor Elias Zintzaras and Professor Georgios Hadjigeorgiou for introducing me to the topic as well as for their support and valuable comments.

I would also like to thank all my Professors at the Postgraduate Programme (MSc) «Research Methodology in Biomedicine, Biostatistics and Clinical Bioinformatics at University of Thessaly».

Many thanks also to my Director and all my colleagues of the ENT Clinic of the General Hospital of Volos “Achillopouleio” for their help and comprehension during the entire time of my post graduate studies.

Last but not least, I would like to thank my family, who have constantly supported me throughout the whole process.

Larissa, September 2016

Nikoleta Batziou

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ABBREVIATIONS

AE	Adverse Event
AZBio	Arizona BioIndustry Association
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
BP	Blood pressure
CEC	Clinical Endpoint Committee
CNC	Consonant-Nucleus-Consonant
CDS	Core Data Sheet
CRF	Case Report Form
CRO	Contract Research Organization
DMC	Data Monitoring Committee
EC	Executive Committee
ECG	Electrocardiogram
EDR	Extemporaneous Dispensing Record
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
HDPT	High Dose Prednisone Taper
ICF	International Classification of Functioning, Disability, and Health
IVRS	Interactive Voice Response System
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IRS	Interactive Response System
ICD	Informed Consent Document
IRB	Institutional Review Board
IEC	Independent Ethics Committee

IB	Investigator Brochure
LPD	Local Product Document
MRI	Magnetic Resonance Imaging
MMSE	Administer Mini Mental State Examination
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
NSAID	Non-Steroidal Anti-Inflammatory Drug
NIDCD	National Institute for Deafness and Communication Disorders
PT	Prothrombin Time
PHQ-8	Administer the Patient Health Questionnaire-8
PTA	Pure Tone Audiometry/Pure Tone Average
RBC	Red Blood Cells
RDA	Recommended Daily Allowance
RCT	Randomized Clinical Trial
SSRS	Suicide Severity Rating Scale
SDS	Speech Discrimination Score
SINT	Speech in Noise Testing
SPL	Sound Pressure Level
SNR	Signal to Noise Ratios
SPC	Summary of Product Characteristics
THC	Tetrahydrocannabinol
USPI	United States Package Insert
WBC	White Blood Cells

CHAPTER 1

INTRODUCTION

1.1 Background and Rationale for the study

Worldwide, hearing loss affects millions of people and can potentially have a negative impact on communication, relationships, and generally the quality of life of patients and their environment (World Health Organization, 2009). There are several types of hearing loss due to a various etiology. This study includes subjects with Sudden Idiopathic Sensorineural Hearing Loss (SSNHL), which pathophysiology usually deal with viral or vascular etiology.

The most common approach to treatment of SSNHL is with steroids and mostly corticosteroids, which has been deemed by many authors to be the gold standard of treatment (Chandrasekhar, 2003).

Adding oral magnesium to the traditional steroid treatment of idiopathic SSNHL may enhance the improvement in hearing. This noninvasive treatment seems to be relatively safe and free of side effects (Nageris et al., 2004).

This trial is designed to study the effectiveness of oral magnesium in combination with oral corticosteroid treatment in patients with unilateral idiopathic SSNHL. Necessary examinations will be performed in order to exclude any other etiology resulting to hearing loss according to exclusion criteria of this protocol.

1.2 Sudden Sensorineural Hearing Loss

The US National Institute for Deafness and Communication Disorders (NIDCD) defines SSNHL as the idiopathic loss of hearing of at least 30 dB over at least 3 contiguous test frequencies

occurring within 72 hours or less (National Institute of Health (2000). The incidence of SSNHL is considered to be 5 to 20 per 100,000 patients per year (Alexander and Harris, 2013). The reported incidence is lowest in patients aged 20 to 30 years (4.7 per 100,000) and highest in those aged 50 to 60 years (15.8 per 100,000) (Byl, 1984). A spontaneous recovery not followed by treatment has been reported to range from 30-60% (Fitzgerald and McGuire, 2007). The fact that several patients experience a spontaneous resolution of their deafness without having seeked treatment, makes the actual incidence higher (Byl, 1977). Sudden hearing loss affects the two sexes equally. Approximately 90% of cases occur unilaterally (Ogasawara et al., 1993). The etiology of SSNHL can be classified into specific categories with multiple conditions within each of these categories, associated with sudden hearing loss:

(1) viral and infectious (meningococcal meningitis, herpes viruses (simplex, varicella zoster), cytomegalovirus, mumps, HIV, mycoplasma, toxoplasmosis, syphilis, measles (rubeola), rubella, Lyme disease.),

(2) autoimmune (lupus erythematosus, polyarteritis nodosa, Cogan's syndrome, Wegener granulomatosis, relapsing polychondritis, Behçet syndrome, Kawasaki disease, temporal arteritis (Horton disease).),

(3) traumatic (perilymphatic fistula, temporal bone fracture, barotrauma, blast injury, etc),

(4) vascular (vertebrobasilar vascular attack, stroke, sickle cell disease, decompression, sickness from SCUBA diving),

(5) neurological (multiple sclerosis, migraine),

(6) tumoral (vestibular schwannoma (acoustic neuroma), leukemia, myeloma, metastasis to internal auditory canal, meningeal carcinomatosis),

(7) Ototoxic (amikacin, vancomycin, erythromycin, cisplatin)

(8) pressure related (hydrops, Ménière's disease).Sudden hearing loss

1.3 Corticosteroids

Oral corticosteroid therapy is the treatment generally accepted and proved to be effective in selected studies and most importantly when compared to placebo in several randomized controlled trials and meta-analyses (Moskowitz et al., 1984; Wilson et al., 1980). Systemic steroids have been labeled the gold standard for treatment of SSNHL, but such a conclusion was based on

a comparison of systemic steroids with placebo (Chandrasekhar, 2003). A burst of steroids such as prednisone is usually administered as a treatment of SSNHL. Regarding the management and steroids for SSNHL, there is a great variability in otology/neurotology practice (Coelho et al., 2011). There has been research on administering preferred oral steroids alone, a combination of oral and intratympanic steroids and also intravenous steroids (Eisenman and Arts, 2000). Evidence to date for a good effect is generally mixed. Two meta-analysis studies of steroid treatment (Conlin and Parnes, 2007; Labus et al., 2010) suggested there was no benefit. In a more recent evidence based review, Lawrence and Thevasagayam (2015) suggested that either oral or intratympanic steroids should generally be offered. Even more recently, Gao and Liu (2016), in another meta-analysis, suggested that combined intratympanic and systemic steroids provide better outcomes than systemic steroids alone (Hain, 2016). On the basis of the study of Wilson et al, oral corticosteroids in moderate doses are the most widely accepted treatment option for idiopathic SSNHL in patients with intermediate audiograms (i.e., neither limited to middle frequency loss nor with profound loss at all frequencies) and this protocol is going to be based on oral corticosteroid treatment (Wilson et al., 1980).

1.4 Magnesium Aspartate

Magnesium is involved in the regulation of cell membrane permeability, neuromuscular excitation, and energy consumption and production (Guenther et al., 1989). A metabolic cellular cascade of events is involved in the putative magnesium mechanism within the auditory system.

Specifically, within the inner ear, magnesium regulates the membrane permeability of the calcium channel in the hair cells, having as a deficiency consequence the over-influx of calcium and sodium, a decrease in potassium by passive diffusion an increased release of glutamate via exocytosis, and overstimulation of N-methyl-D-aspartate receptors on the auditory nerve fibers (Attias et al., 1994). This diminishes the electrochemical gradient required for sensory transduction and attempting to restore this gradient, the hair cells expend energy, which can ultimately lead to cell death (Attias et al., 1994).

This lack of appropriate magnesium intake may have negative consequences in hearing. An extracellular magnesium deficiency may also lead to the release of hormones that decrease muscle tone, catecholamine and prostaglandin secretion, thereby with subsequent vasoconstriction and a reduction of blood flow to the cochlea (Thorne and Nuttall, 1987; Günther et al., 1978)

Metabolic stress within the inner ear, such as a possible noise exposure also places high demands on cell energy stores (Spoendlin, 1962). The exact mechanism by which magnesium supplementation works remains unclear. However, it is speculated that supplementation increases magnesium levels within the inner ear to maintain normal membrane characteristics and cell energy demands. Increased magnesium levels are also thought to improve the microcirculation of the inner ear, therefore, improving blood flow to the cochlea (Altura et al., 1992)

Recent studies of both noise-induced hearing loss and idiopathic sensorineural hearing loss have suggested that magnesium supplementation may even lessen the severity of tinnitus in patients. Magnesium improved hearing recovery and lessened tinnitus in patients with idiopathic sudden hearing loss (Gordin et al., 2002). More recently, Nageris et al. (2004) showed in a controlled study that magnesium was a relatively safe and convenient adjunct to corticosteroid treatment for enhancing the improvements of hearing in acute-onset sensorineural hearing loss. The protective effect of magnesium in noise-induced hearing loss has been previously reported whereas the therapeutic is still on research (Ising et al., 1982; Scheibe et al., 2000).

1.5 Rationale for Dose Selection

The Recommended Daily Allowance (RDA) for magnesium in adults is 4.5 mg/kg (Saris et al., 2000); however, according to studies on Americans, we usually fall short of the RDA for magnesium by 100 mg daily (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997).

Taking into consideration results of the trials of Nageris et al. (2004) and Slattery et al. (2005) treatment studies, that showed that a 14-day course of oral prednisone 60 mg per day to be approximately comparable for efficacy and safety, following a taper therapy treatment for one more week aiming to a gradual decrease of two daily doses of 20 mg of prednisone for 3 days and one daily dose of prednisone for 4 days. Retrospective reviews found patients who were treated within two weeks of symptoms with at least 60 mg prednisone daily did better than patients who received lower doses of glucocorticoid, or who were treated later in their course (Slattery et al., 2005).

In our study protocol, the magnesium aspartate dose was selected to be 167mg in 200ml of lemonade per mouth once daily.

As placebo, sodium aspartate 167mg in 200ml of lemonade will be administered for oral intake once daily (Nageris et al., 2004; Slattery et al., 2005).

1.6 Preclinical Safety Data

Corticosteroids may cause hypernatremia, hypokalemia, fluid retention, and elevation in blood pressure. These mineralocorticoid effects are most significant with fludrocortisone, followed by hydrocortisone and cortisone and finally by prednisone and prednisolone. The remaining corticosteroids, betamethasone, dexamethasone, methylprednisolone, and triamcinolone, have minor reported mineralocorticoid activities. However, large doses of any corticosteroid can demonstrate these effects, particularly if given for longer than brief periods. Therapy with corticosteroids should be administered cautiously in patients with preexisting fluid retention, hypertension, congestive heart failure, and/or renal dysfunction. Dietary sodium restriction and potassium supplementation may be advisable (Klepikov et al., 1988).

Mg2+ has several vital functions in the human body. The main body source of Mg2+ is the skeletal system (70%), whereas the serum contains only 0.3%. The main regulator of the Mg2+ levels is the kidney, which secretes it whenever there is an excess and reabsorbs when there is a deficiency. Hormones such as calcitonin, aldosterone, thyroxin, and insulin influence Mg2+ balance. Magnesium is likely safe for most people when taken by mouth appropriately or when the prescription-only, injectable product is used correctly. In some people, magnesium might cause stomach upset, nausea, vomiting, diarrhea, and other side effects, such as mild hypotension. Doses less than 350 mg daily are safe for most adults (Schanne et al., 1979).

1.7 Clinical Safety Data

Positive findings were noted in guinea pigs that were given magnesium injections immediately after use of the transient threshold shift strategy (Scheibe et al., 2000).

It has been recently demonstrated in Randomized Clinical Trials (RCTs) results that a preventive Mg2+ supplement can reduce ischemia-induced hearing loss and serum level of Mg2+ is not an indicator of perilymphatic Mg2+ levels. The perilymphatic level may be low while the serum level is normal and most SSNHL patients have a normal range of serum Mg2+ levels. A

more reliable correlation may be found between perilymphatic and intracellular Mg2+ levels (Scheibe et al., 2000; Attias et al., 1994).

Mg2+ treatment combined with carbogen in patients with SSNHL showed to improve significantly the hearing recovery compared with carbogen treatment alone. According to the literature, it is reported that spontaneous recovery in patients with SSNHL is about 50 to 70%. The results may also differentiate according to the criteria for recovery of each study (Gordin et al., 2002). Nageris et al. (2004) used a prospective, randomized, double-blind placebo controlled trial to investigate the effectiveness of oral magnesium supplementation in the treatment of SSNHL. A significantly greater rate of recovery was reported among patients treated with steroids plus magnesium (n = 14) vs steroids plus placebo (n = 14) as measured 4 weeks after treatment. Group data analysis showed that the treatment group had a greater proportion of subjects with improved hearing thresholds at all test frequencies ($F=4.8$, $p<0.02$) and an overall greater mean improvement ($F=3.7$, $P<0.05$). Overall, according to these results it is suggested that magnesium supplementation can enhance steroid treatment of SSNHL.

Gordin et al. (2002) completed a prospective, randomized, placebo-controlled trial to investigate the effectiveness of magnesium treatment for improving the outcome of unilateral SSNHL. There were 278 subjects diagnosed with SSNHL who were randomly assigned to either the control group, treated with carbogen inhalation or to the treatment group, treated with intravenous magnesium and carbogen inhalation. Subjects were later excluded from the control group for additional treatment (n=20) and from the treatment group due to side effects associated with magnesium (n=10) and several subjects were also lost from the control (n=59) and the treatment group (n=56) during follow-up procedures. Treatment continued until hearing was determined to be normal or similar to the better hearing ear using standard audiometric testing.

For the purpose of their study, Gordin et al. (2002) defined recovery as an improvement rate greater than 75%. Recovery was achieved in 48% of the treatment group and 31.6% of the control group ($p<0.01$) and the mean improvement rate was reported as 66.4% in the treatment group and 49.9% in the control group ($p<0.01$). Overall, these results suggest that magnesium treatment may improve recovery in cases of SSNHL.

Attias et al. (1994) conducted a prospective, randomized, double-blind, placebo-controlled trial to investigate the prophylactic value of long-term oral intake of magnesium in reducing noise-induced permanent threshold shift. Male subjects (n=300) were randomly assigned to either the

control group, receiving a daily oral placebo treatment or to the treatment group receiving a daily oral magnesium supplement. All subjects experienced a similar lifestyle in terms of noise exposure, diet, and daily routine. Results revealed that the placebo group had significantly more frequent and severe permanent threshold shifts ($x2=8.1$, $p<0.001$). Overall, these results suggest magnesium supplementation may reduce noise-induced permanent hearing damage.

Finally, Attias et al. (2004) evaluated the effects of magnesium intake on temporary threshold shift carrying a prospective, double-blind crossover study. Normal-hearing male subjects (n=20) participated in three measurement sessions: (1) a baseline measurement prior to any treatment, (2) a measurement 10 days after daily oral magnesium treatment, (3) a measurement 10 days after daily placebo treatment. All subjects of the study were exposed to 90 dB Sound Pressure Level (SPL) white noise for a 10minute duration and audiometric evaluations were conducted prior to and after noise exposure until thresholds returned to normal. The results of this study suggest that magnesium supplementation can reduce noise-induced temporary threshold shift (Nageris et al, 2004; Gordin et al., 2002; Attias et al., 2004).

CHAPTER 2

TRIAL OBJECTIVES AND HYPOTHESES

2.1 Objectives

Primary

- ◊ To evaluate efficacy of magnesium in combination with corticosteroids in the treatment of idiopathic sudden sensorineural hearing loss based on patients' auditory system status.
- ◊ To evaluate the safety and tolerability of magnesium in the treatment of idiopathic sudden sensorineural hearing loss.

Secondary

- ◊ To assess the efficacy of magnesium in combination with corticosteroids in the treatment of idiopathic sudden sensorineural hearing loss using the following methods
  - o Laboratory blood tests.
  - ◊ Pure-tone threshold for air and bone conduction.
  - ◊ Pure-tone average (PTA).
  - ◊ Speech Discrimination Score (SDS).
  - ◊ Speech In Noise Testing (SINT).

◊ Tinnitus Severity Ranking Scale.

(Gordin et al., 2002; Hain, 2016; Bednar et al., 2015)

2.2 Endpoints

Primary

Efficacy:

◊ Change from baseline to Tmax in Pure Tone Audiometry (PTA) averaged over 2 and 4 kHz; measured in the ear with the greater hearing deficit (determined at baseline).

Safety:

◊ Adverse Events, ECG, vital signs, safety laboratories.

Secondary

Efficacy:

◊ Change from baseline in PTA averaged over 2 and 4 kHz;

◊ Measured in the ear with the greater deficit (determined at baseline).

◊ Change from baseline in SDS (Speech Discrimination Score) measured in the worst hearing ear as defined by PTA at baseline.

◊ Change from baseline in SINT.

◊ Change from baseline in Tinnitus Severity Ranking Scale

(Bednar et al., 2015).

CHAPTER 3

TRIAL DESIGN

This is a randomized, double-blind, placebo-controlled clinical trial assessing the therapeutic effect of magnesium aspartate, along with parallel administration of corticosteroids regarding patients diagnosed with unilateral idiopathic sudden sensorineural hearing loss. Subjects will receive 167 mg of magnesium aspartate or sodium aspartate (placebo) in 200ml of lemonade whereas both groups will be treated with 60 mg of corticosteroids per day, divided in three doses, for 15 days and taper therapy for one more week (Levie et al., 2007; Nageris et al., 2004).

3.1 Details for the design of the trial

The study will be divided into a screening period, a double-blind treatment period closing with a study end visit, and a post-treatment observation period. At the study end visit or at an early study medication discontinuation visit for premature discontinuation of study therapy, subjects will be transitioned from 60 mg of prednisone daily dosage to taper therapy for one week-decreasing dose 20 mg every 3 days. Patients may discontinue study drug for any of the following reasons: safety concerns, pregnancy, elevation of blood glucose levels on two consecutive measurements, severe hypotension or hypertension, noncompliance, or the need for an excluded medication.

The time on study drug will vary from subject to subject depending upon the time of the subject's enrollment. Subjects will be qualified for the study within 2 days before randomization to allow adequate time for the site to review the inclusion and exclusion criteria for the prospective study participant. After meeting all study entry criteria, subjects will be randomized into treatment

groups and begin study drug treatment. The randomization will take place with the use of an Interactive Voice Response System (IVRS) and subjects will be randomly assigned in a 1:1 ratio to 1 of the following 2 treatment groups:

◊ Magnesium aspartate 167mg in 200ml of lemonade per mouth once daily plus prednisone 60 mg per mouth divided in 3 daily doses of 20 mg for 2 weeks, following one more week of gradual decrease-two daily doses of 20 mg of prednisone for 3 days, one daily dose of prednisone for 4 days.

OR

◊ Placebo sodium aspartate 167mg in 200ml of lemonade per mouth once daily plus prednisone 60 mg per mouth divided in 3 daily doses of 20 mg for 2 weeks, following one more week of gradual decrease-two daily doses of 20 mg of prednisone for 3 days, one daily dose of prednisone for 4 days (Attias et al., 1994).

Once the subject's eligibility for the study has been confirmed, the subject will after be randomized and study drug will be dispensed. A 12-lead electrocardiogram (ECG), blood pressure measurement, audiometry and clinical laboratory tests will be performed every three days for the treatment period of 3 weeks. Subjects will return for visits after 4 weeks of the discontinuation of medication.

All randomized subjects will be followed until the study ends (adjudicated endpoint events reached followed by study closure activities) even if they did not take study drug or prematurely discontinued study drug. Every effort should be made to contact any subjects lost in order to follow-up and collect information on the occurrence of efficacy endpoint events and the reason for discontinuation. When the pre-specified number of adjudicated primary efficacy endpoint events has been reached in the per protocol population, the sites will be notified by the sponsor to schedule each subject still receiving blinded study medication for a study-end-visit.

The post-treatment observation period ends with a follow-up visit, which will be performed approximately 30 days after the study-end-visit. Subjects who have previously prematurely discontinued study drug will be contacted for a final assessment of efficacy endpoint events within 30 days of site notification.

An Executive Committee (EC) will be formed that has the overall responsibility for the conduct and reporting of the study. An independent Data Monitoring Committee (DMC) will be commissioned for this study, in order to monitor the progress of the study and to ensure that the safety of the subjects is not compromised. An independent blinded Clinical Endpoint Committee

(CEC) will apply the protocol definitions and adjudicate and classify the endpoints (Bednar et al., 2015).

3.2 Committees

3.2.1 Executive Committee

The EC consists of academic leadership members and one member from the possible sponsoring company. The EC will ultimately be responsible for the conduct of the study including addressing any Data Monitoring Committee recommendations and overseeing publication according to the results (Bednar et al., 2015).

3.2.2 Steering Committee

A Steering Committee will be formed consisting of members who are the lead investigators from each region. The Steering Committee will advise and assist the EC with regard to the scientific and operational aspects of the study (Bednar et al., 2015).

3.2.3 Independent Data Monitoring Committee

This study will be conducted under the auspices of an independent Data Monitoring Committee (DMC), which will ensure the safety of subjects enrolled in the study and monitor the progress of the trial. The DMC will consist of a chairperson, two otorhinolaryngologists, as well as a statistician. This committee will review on a regular basis the accumulating data, and may also request to review partially or totally unblinded accumulating data. The DMC will make recommendations to the Executive Committee regarding the continuing safety of subjects currently enrolled and yet to be enrolled in the trial. During the whole course of the study, the DMC may request access to unblinded data if needed (Bednar et al., 2015).

3.2.4 Clinical Endpoint Committee

The Clinical Endpoint Committee (CEC), composed of experts in the relevant fields, will review, in a blinded manner, all reported study outcomes in order to provide consistency and validity in the assessment of outcomes. Their decisions will be based on blind clinical data and will be used for the final statistical analyses (Clinical Research Resource HUB, 2015).

CHAPTER 4

ETHICAL CONSIDERATIONS

4.1 Institutional Review Board (IRB)/ Independent Ethics Committee (IEC)

It is the investigator's responsibility to have prospective approval of the study protocol, protocol amendments, informed consent documents, and other relevant documents, such as recruitment advertisements, if applicable, from the IRB/IEC. All correspondence with the IRB/IEC should be retained in the Investigator File.

The only circumstance in which an amendment may be initiated prior to IRB/IEC approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the investigator must notify the IRB/IEC (Bednar et al., 2015).

4.2 Subject Information and Consent

Protection of subject personal data has to be strictly ensured and so there will not be included subject names on any sponsor forms, reports, publications, or in any other disclosures, except where required by laws.

Subject names, address, birth date and other identifiable data will be replaced by a numerical code consisting of a numbering system in order to de-identify the trial subject. The informed consent document must be in compliance with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP), local regulatory requirements, and legal requirements.

The informed consent document used in this study, and any changes made during the course of the study, must be prospectively approved by the IRB/IEC.

The investigator must ensure that each study subject, or his/her legal representative is fully informed about the nature and objectives of the study and possible risks associated with participation.

The investigator will obtain written informed consent from each subject or the subject's legal representative before any study-specific activity is performed (Bednar et al., 2015).

4.3 Subject Confidentiality

Confidentiality must be preserved by investigators and all subjects taking part in the study, in accordance with GCP and local regulations.

The Investigator must ensure the subject's anonymity. On the electronic Case Report Forms (eCRFs) or other documents submitted to the Contract Research Organization (*CRO*), subjects should be identified by a unique subject identifier (Bednar et al., 2015).

4.4 Subject Recruitment

It is possible to use advertisements, approved by ethics committees and investigator databases, as recruitment procedures (Bednar et al., 2015).

4.5 Ethical Conduct of the Study

The study will be conducted in accordance with all legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences [CIOMS], 2002; Vijayananthan and Nawawi, 2008; World Medical Association, 2008).

In addition, the study will be conducted in accordance with the protocol, the ICH guideline on GCP, and applicable local regulatory requirements and laws.

4.6 Reporting of Safety Issues and Serious Breaches of the Protocol or ICH and GCP

In the event of any prohibition or restriction by an applicable Competent Authority, or if the investigator is aware of any new information which might influence the evaluation of the

benefits and risks of the investigational product, the possible sponsor company should be informed immediately.

In addition, a written report should immediately be prepared by the investigator, in case of any urgent safety measures taken in order to protect the study subjects against any immediate hazard, and of any serious breaches of this protocol or of ICH and GCP that the investigator may become aware of (Bednar et al., 2015).

CHAPTER 5

SUBJECT SELECTION

There are certain eligibility criteria designed to select subjects for whom protocol treatment can be considered appropriate in order to fulfill the study objectives. All relevant medical and non-medical conditions should be taken into consideration.

5.1 Inclusion Criteria

The subjects' eligibility criteria must be appropriately examined, reviewed and documented by a properly qualified member of the investigator's study team before getting included in the study.

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Evidence of a personally signed and dated informed consent document indicating the subject awareness of all pertinent aspects of the study.
2. Subjects who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, efficacy assessments, safety assessments, and other study necessary procedures.
3. Male, and female subjects of non-childbearing potential who are 20-65 years of age and a body weight of at least 50 kg.
4. Subjects who can read, speak and comprehend Greek or English.

5. Subjects must have a current diagnosis of unilateral idiopathic sudden sensorineural hearing loss which has occurred within the last 72 hours in the range of 30-60 dB (inclusive), averaged over 2 and 4 kHz in at one ear. The Pure Tone Audiometry (PTA) assessment will provide the basis of the diagnosis.

6. Subjects must have symmetric hearing loss with respect to both PTA and Speech Discrimination score (SDS).

7. Subjects who are hearing aid users will be allowed but subjects will refrain from wearing the hearing aid on the day of drug administration and for at least 24 hrs after procedure visits (Bednar et al., 2015; Plontke et al., 2009).

5.2 Exclusion Criteria

Subjects presenting with any of the following will be excluded from the study:

1. Pregnant females and breastfeeding females.

2. Subjects with a history of sudden hearing loss and diagnosis of rapidly progressive idiopathic hearing loss.

3. Subjects who have the following hearing disorders or any other hearing disorders that may impact efficacy assessments or safety of subjects in the opinion of the investigator: Meniere's disease (history or present), concurrent vestibular pathology, radiation-induced hearing loss, endolymphatic hydrops, suspected retro-cochlear lesion or fluctuating hearing loss; history of asymmetric hearing loss, conductive hearing loss (defined as Bone Conduction – Air Conduction), prior ear surgery (except ventilating tubes), congenital hearing loss, genetic SSNHL with strong family history, active otitis externa or media, cochlear ossification, cholesteatoma or otosclerosis.

4. Subjects with moderate or greater tinnitus as indicated by a Tinnitus Handicap Inventory Score of 38.

5. Subjects with a speech discrimination score obtained at the screening visit of <60% in either ear.

6. Subjects with systemic diseases that could affect hearing or hearing assessment, such as a history of autoimmune disease (e.g. rheumatoid arthritis, lupus, etc.), myasthenia Gravis, myopathy, dementia, hyperadrenocorticalism, hyperlipidemia, electrolyte imbalance, osteoporosis, (+) tuberculin test.

7. Subjects on prohibited medications and not able to discontinue these concomitant medications after the screening visit. Subjects taking concomitant-medications that can effect hearing, including but not limited to systemic steroids, aminoglycosides, ototoxic chemotherapeutic drugs taken at any time in the past (e.g., cis-platinum). Subjects taking concomitant-medications that may affect the pharmacokinetics of the drug will be excluded.

8. Current (past 2 weeks prior to screening) pathological noise exposure either due to occupational, recreational, or other types of noise exposure (e.g. 1) occupational noise in professions requiring regular use of hearing protection, 2) target shooting/firing range exposure, 3) hunting, etc.).

9. Subjects with a cochlear implant.

10. Subjects with evidence or history (referring to the current timeframe) of clinically significant cardiovascular disease, including uncontrolled hypertension (sitting or supine diastolic pressure >95 mm Hg and/or sitting or supine systolic pressure >170 mm Hg with or without treatment), hypotension, bypass surgery, history of myocardial infarction or ischemic heart disease, uncompensated heart failure or recent acute myocardial infarction, as determined by the investigator. Controlled essential hypertension and non-clinically significant sinus bradycardia and sinus tachycardia will not be considered significant medical illnesses and would not exclude a subject from the study.

11. Subjects with a medical history of glaucoma, ocular herpes simplex and ototoxicity.

12. Subjects who are known to have AIDS or to be HIV positive.

13. Subjects with screening laboratory test results that deviate from the upper or lower limits of the reference range, except for not clinically significant values, as determined by the investigator.

14. Subjects with a 12-lead ECG with repeated demonstration of QTcF >450 msec at screening or at baseline (Day 1) based on the automated machine reading.

15. Any condition possibly affecting drug absorption (e.g., gastrectomy).
16. For subjects with a high risk assessment done by a qualified mental health professional to assess whether it is safe for the subject to participate in the study. In addition, subjects deemed by the investigator to be at significant risk of suicidal or violent behavior should be excluded.
17. Subjects who have taken an investigational drug within 30 days prior to baseline.
18. Participation in other interventional/investigational drug studies within 30 days before the current study begins and/or during study participation.
19. Blood donation in the 60 days prior to the screening visit or any subject planning to donate blood during the course of the clinical trial.
20. Subjects who have presbycousis.
21. Unwilling or unable to comply with the Life Style Guidelines described in this protocol.
22. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the subject inappropriate for entry into this study.
23. History of regular alcohol consumption exceeding 14 drinks/week (1 drink = 5 oz. of wine [150mL] or 12 oz. [360 mL] of beer or 1.5 oz. [45 mL] of hard liquor) within 6 months of Screening.
24. Subjects who are smokers whose smoking pattern would prevent them from completing the Screening or Treatment period assessments without nicotine withdrawal symptoms.
25. Subjects who are investigational site staff members or their relatives or subjects who are directly involved in the conduct of the trial.

(Bednar et al., 2015; Plontke et al., 2009).

5.3 Life Style Guidelines

5.3.1 Meals and Dietary Restrictions

It is necessary that subjects will have abstained from food and drink at least 3 hours prior to the Screening safety laboratory evaluations. There is a dietary restriction regarding sugar and salt.

5.3.2 Alcohol, Caffeine and Tobacco

Subjects must abstain also from alcohol for 24 hours prior to admission to the study site. Alcohol consumption will not exceed 14 drinks/week throughout the study. Subjects may undergo an alcohol breath test at the discretion of the investigator. Subjects will limit caffeine in coffee, tea and/or other caffeine-containing beverages, food or drugs to no more than an average of 300 mg of caffeine/day within observation and treatment periods (~2-3 cups coffee, ~6 cans soda). Subjects will abstain from the use of tobacco- or nicotine-containing products for 3 hours prior to dosing (Bednar et al., 2015).

5.3.3 Activity

Subjects must abstain from strenuous exercise for at least 48 hours prior to blood collection for clinical laboratory tests for all visits (Bednar et al., 2015).

CHAPTER 6

RANDOMIZATION AND BLINDING

6.1 Randomization and Blinding

Central randomization will be implemented in conducting this study. At the time of enrollment, each subject will be assigned a unique sequential subject number by the IVRS (interactive voice response system) and a treatment code, which will dictate the treatment assignment for that subject. The IVRS will be available 24 hours per day, seven days a week. The subject number will consist of a unique 5 digit number which is assigned sequentially within the study (starting with 00001) by the IVRS. This number will be used for identification throughout the study and will not be used for any other subject. Subjects will be randomly assigned in a 1:1 ratio (magnesium aspartate plus prednisone to sodium aspartate plus prednisone) to either treatment or control groups and the randomization will be stratified by age groups and first audiometric results. So the IVRS will then also assign a medication kit (and subsequent medication kits) that matches the treatment code to which the subject has been randomized.

The investigator will not be provided with randomization codes. The codes will be maintained within the IVRS, which has the functionality to allow the investigator to break the blind for an individual subject.

This study has a double-blind design. Neither the subjects nor any of the Investigators or Sponsor staff involved in the treatment or clinical evaluation of the subjects will be aware of the treatments received. There will be an independent DMC to monitor the data (audiometry, laboratory tests) in an unblinded manner on a periodic basis. An independent statistician, not otherwise involved in the study, will prepare and provide the required reports to the DMC.

The blind should be broken only if specific emergency treatment would be dictated by knowing the treatment status of the subject. If for any reason, the Investigator needs to become unblinded to the treatment of a subject, he/she will make every attempt to first call the sponsor and discuss the need for unblinding and obtain agreement. If the investigator is unable to contact the sponsor, the investigator may in an emergency determine the identity of the treatment by telephoning IVRS. The sponsor must be informed as soon as possible by the investigator. Efforts should be made to limit access to knowledge of the treatment assignment to only those individuals who need to know the information and the subject should continue in the study.

Magnesium aspartate and its matching placebo will be taken once daily as a fixed dose of 200 ml of lemonade containing 167 mg of magnesium or sodium aspartate respectfully. Prednisone will be administered to both study groups in three daily doses of 20mg. The age and first audiometry results will be embedded in a “code number” and reported to a central IVRS along with the subject's study identification number.

CHAPTER 7

STUDY PROCEDURES AND ASSESSMENTS

In cases that circumstances, outside of the control of the investigator, may make it unfeasible to perform the tests, the investigator will take all steps necessary to ensure the safety and the well-being of the subjects. When a protocol required test cannot be performed the investigator must document the reasons for this and any corrective and preventive actions which he/she has taken to ensure that normal processes are adhered to as soon as possible.

Blood Volume

Total blood sampling for the individual subjects is approximately 134 ml. Additional blood samples may be taken for safety assessments and the IRB is notified of the blood collection.

7.1 Screening Period

Subjects need to be screened prior to administration of the study medication in order to confirm that they meet the subject selection criteria for the study. The investigator will obtain informed consent from each subject according to the procedures described.

The following procedures must be completed (Bednar et al., 2015; Lawrence et al., 2015; Schweinfurth et al., 1997; Nageris et al., 2003):

- ◊ Obtain written informed consent.
- ◊ Review inclusion and exclusion criteria.

◊ Complete medical history including neurological, psychiatric and hearing loss history.

◊ Full physical and neurological examination.

◊ Height and weight.

◊ Complete history of all prescription or nonprescription drugs, and dietary and herbal supplements taken within 28 days prior to the planned first dose.

◊ History of drug, alcohol and tobacco use.

◊ Review history of medical devices including hearing aid use.

◊ Verify noise exposure within the past 2 weeks due to occupational, recreational, or other types of noise exposure including but not limited to jackhammers, jet engines, firing ranges, etc.

◊ Oral temperature.

◊ Supine and standing blood pressure and pulse rate.

◊ Singlet 12-lead electrocardiogram (ECG).

Following at least a 3-hour fast, blood and urine specimens will be collected for the following:

◊ Safety laboratory tests (chemistry, hematology and urinalysis).

◊ Urine drug screen.

◊ Administer Mini Mental State Examination (MMSE).

◊ Administer the Patient Health Questionnaire-8 (PHQ-8).

◊ Suicidality Monitoring: administer Columbia Suicide Severity Rating Scale (C-SSRS) Baseline/Lifetime Assessment.

◊ Administer Tinnitus Handicap Inventory before audiology assessments.

◊ Perform Pure Tone Average (PTA) assessment.

◊ Speech Discrimination Score (SDS).

◊ Brain Magnetic Resonance Imaging (MRI) to exclude the presence of tumors such as an acoustic neuroma.

Before any audiometric assessments the audiologist will first examine the ears with an otoscope and in case that significant cerumen is occluding the canal, the cerumen must be removed prior to study participation. All the potential subjects will have the study risks and benefits explained in detail to them, the associated International Classification of Functioning, Disability, and Health (ICF) reviewed with them, and all questions answered for them. Screening procedures will be performed within 2 days before randomization. The investigator should obtain relevant medical history and vital signs and perform physical examination. The results of all screening procedures must be reviewed before randomization to ensure that all inclusion criteria and none of the exclusion criteria are met.

7.2 Treatment period

Subjects entering the study will be administered orally 20mg of prednisone tablets 3 times per day, for 2 weeks. The last dose will not be administered later than 8 pm. After the first two weeks of the study High Dose Prednisone Taper (HDPT) therapy will follow for one more week. Laboratory tests, vital signs and audiometric examination will be followed every three days of the study.

7.3 Post-treatment observation period- follow-up visit

After the study end visit or Study drug discontinuation visit, there will be an observation period to follow subjects after transition. Subjects will be asked to return to the clinic for a follow-up visit approximately 30 days after the permanent discontinuation of study drugs.

At the follow-up visit the Investigator will:

- ◊ Assess for Serious Adverse Events (SAEs) (until 30 days after the last dose of double-blind study drugs).
- ◊ Assess for outcomes (laboratory test results, audiometric results).

7.4 Subject Early Termination/Withdrawal

It is possible for subjects to withdraw from the study at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety or behavioral reasons, or the inability of the subject to comply with the protocol required schedule of visits and procedures. In case of a subject does not return for a scheduled visit, every effort should be made to contact the subject. The investigator should inquire about the reason for withdrawal, request the subject to return all unused investigational product(s), request the subject to return for a final visit, if applicable, and follow-up with the subject regarding any unresolved adverse events.

If a subject withdraws during a treatment period, the subject should be scheduled for a final follow up visit within 30 days of the last dose of study treatment. Assessments may include:

- ◊ Obtain information regarding the use of concomitant medications.
- ◊ Brief neurological examination.
- ◊ Collect a blood sample for pharmacokinetic.
- ◊ Verification of noise exposure within the past 2 weeks due to occupational, recreational, or other types of noise exposure including but not limited to jackhammers, jet engines, firing ranges, etc.
- ◊ Supine and standing blood pressure and pulse rate.
- ◊ Safety laboratory tests (chemistry, hematology and urinalysis).
- ◊ Assess symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as “How do you feel?”
- ◊ Discharge from study.

If the subject withdraws from the study, and also withdraws consent for disclosure of any future information, no further evaluations should be performed, and no additional data should be collected. Subjects who withdraw from the study can be replaced at the according to the investigator upon consultation with the sponsor (Bednar et al., 2015).

7.5 Efficacy Assessments

7.5.1 Pure Tone Audiometry/Pure Tone Average (PTA)

The most common procedure for the measurement of hearing loss is pure tone audiometry is. The tones with a single frequency of vibration, which are called pure tone signals, are delivered to the subject via insert earphones and a bone vibrator. At the screening visit, air and bone conduction audiometry will be performed. Air conduction audiometry will be performed with insert earphones and thresholds will be obtained at 0.25, 0.5, 1, 2, 4, and 8 kHz. Bone conduction audiometry will assess thresholds at 0.5, 1, 2, and 4 kHz tones. The air-bone gap, which is defined as the comparison of air-conduction and bone-conduction thresholds, helps to characterize the type of hearing loss. For study visits, only air conduction audiometry will be performed at baseline and at post-dosing assessments. Audiometric thresholds will be assessed the times specified in this protocol (Bednar et al., 2015).

7.5.2 Speech Discrimination Score (SDS)

The Speech Discrimination Score (SDS) presents monosyllabic words, phonetically balanced at above the speech reception threshold (SRT) intensities or a Pure Tone Average (PTA) of 0.5, 1, and 2 kHz tones and measures speech comprehension through. Maximal speech discrimination scores are typically seen at SRT or PTA plus 25-40 dB. Results are expressed as a percentage of words repeated correctly. For the Screening assessments only, the SDS will be measured independently in the right and left ear using one-half of a 50-word list of Consonant-Nucleus-Consonant (CNC) words presented at 25-40 dB above the pure tone average of 0.5, 1, and 2 kHz tones. The level of presentation will be based on the audiologist's discretion regarding subject comfort level. Subjects with SDS scores in either ear of <60% at screening will be ineligible for the study. Also, SDS should be symmetric, meaning that the difference in SDS between ears should be <20%, since a greater difference may be associated with pathophysiological processes that could affect efficacy endpoints (Bednar et al., 2015).

7.5.3 Speech in Noise Testing (SINT)

A direct measurement of functional speech perception in noise in the sound field utilizing both ears at once will be performed using Arizona BioIndustry Association (AZBio) Sentences. AZBio scores will only be measured under the condition of Multitalker Babble noise presented from the front. Each subject will be administered one list of AZBio Sentences in Quiet at 60 dB SPL. The % correct score is computed and half of that will be denoted as a Target Score. Testing is continued at various signal to noise ratios (-10, -7, -4, -1, +2, +5, +8, +11) in which the speech input is kept constant at 60 dB SPL, and the Multitalker babble noise is set to the test ratio to determine the Target Signal to Noise Ratios (SNR) (the level at which the subject obtains a percent correct score within 9% of the Target Score). Each test repetition at a particular SNR will involve the use of one list of AZBio sentences. There are 8 lists of AZBio Sentences which will be administered at baseline and two specific time points at each period. To reiterate subjects will be administered one list of AZBio Sentences in Quiet at 60 dB SPL which will be used to generate the Target Score. Testing will continue at various SNR in which the speech input is kept constant at 60 dB SPL, and the Multitalker babble noise is set to the test ratio to determine the Target SNR. The SINT will be assessed as per times specified in this protocol (Bednar et al., 2015).

7.5.4 Tinnitus Handicap Inventory

The Tinnitus Handicap Inventory is a self-report questionnaire that lists subjective questions designed to identify problem areas that may be present if a subject is distressed by the possible presence of tinnitus. Questions include targeting of emotions (i.e., depression, anxiety), limitations and generally the inability to cope. The Tinnitus Handicap Inventory will be assessed before the subject performs the audiology assessments at the Screening visit. The Tinnitus Handicap Inventory will be assessed as specified in this protocol (Bednar et al., 2015).

7.5.5 Tinnitus Severity Ranking Scale

The Tinnitus Severity Ranking Scale is a self-report measure of tinnitus distress severity utilizing a scoring system from 1-10 to reflect changes in tinnitus distress over time (Bednar et al., 2015).

7.6 Safety

7.6.1 Laboratory

The following safety laboratory tests will be performed at the time points specified in this protocol:

- ◊ Hemoglobin
- ◊ Hematocrit
- ◊ RBC count
- ◊ Platelet count
- ◊ WBC count
- ◊ Total neutrophils
- ◊ Eosinophils
- ◊ Monocytes
- ◊ Basophils
- ◊ Lymphocytes
- ◊ MCH
- ◊ MCHC
- ◊ MCV
- ◊ Reticulocyte count
- ◊ Creatinine
- ◊ Glucose
- ◊ Urea
- ◊ Ca++
- ◊ Na+, K+, Cl-
- ◊ Total CO2 (Bicarbonate)

◊ AST, ALT

◊ Total Bilirubin

◊ Alkaline phosphatase

◊ Uric acid

◊ Albumin

◊ Total protein

◊ Creatine Kinase

◊ pH

◊ Urine Drug Screen

Safety laboratory tests from Screening, as judged by the Investigator, must have no clinically significant findings, in order for a subject to be enrolled in the study.

Urine drug screening will be performed with minimum requirement for drug screening including: cocaine, Tetrahydrocannabinal (THC), opiates, benzodiazepines and amphetamines. Subjects may undergo random urine drug testing at the discretion of the investigator. Drug testing conducted prior to dosing must be negative for subjects to receive study medication (Bednar et al., 2015).

7.6.2 Blood Pressure and Pulse Rate

Blood pressure (BP) and pulse rate will be measured at certain time points. Supine blood pressure will be measured with the subject's arm supported at the level of the heart, and recorded to the nearest mm Hg after 5 minutes of rest. The same arm will be used throughout the study (Bednar et al., 2015).

◊ Single supine and standing blood pressure and pulse rate will be measured at times specified in this protocol.

◊ Subject in supine position for a minimum of 5 minutes.

◊ Stand subject up for 3 minutes.

◊ Assess BP immediately after 3 minutes in standing position.

7.6.3 Electrocardiogram (ECG)

A triplicate and single 12-lead ECG will be collected as scheduled in this protocol. All scheduled ECGs should be performed after the subject has rested quietly for at least 10 minutes in a supine position. The investigator is responsible for reviewing machine interpretations and for retaining hard copies of the ECG tracings and reports and the documentation of time and date of the ECG performance. Any clinically important findings will be noted in the CRF. The centrally reported ECG results will be used in the study analysis. In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality (Bednar et al., 2015).

7.6.4 Physical Examination

The complete physical examination will evaluate any clinically important abnormalities within the body systems (e.g., skin, head/eyes/ears/nose/throat, neck, heart, lungs, abdomen, musculoskeletal system and lymph nodes) (Bednar et al., 2015).

7.6.5 Neurological Examination

A full neurologic examination will be completed including level of consciousness, speech, cranial nerves (including pupil equality and reactivity), motor function, sensory function, coordination, gait, and reflexes. Identification of any pre-existing movements should be emphasized for comparison to post-dosing examinations. Observation for movements should focus on an approximately 15 second observation period for any given anatomic area. Clinically important abnormalities and any movements observed at the screening visit should be noted on source documents and/or the medical history.

The brief neurological exam will include observation for cerebellar (intention) tremor and for non-cerebellar tremors (resting), finger nose, heel shin, Romberg, tandem walking, and spontaneous and gaze-evoked nystagmus (Bednar et al., 2015).

7.6.6 Mini Mental State Examination (MMSE)

The MMSE is a brief, widely used test for assessing overall cognitive state. The MMSE measures selected aspects of cognition such as memory, orientation, attention, language, and praxis on a scale of 0 to 30. Lower scores indicate greater cognitive impairment. The MMSE will be performed only during Screening (Bednar et al., 2015).

CHAPTER 8

ADVERSE EVENT REPORTING

8.1 Adverse Events

For all Adverse Events (AEs), the investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets the criteria for classification as an SAE requiring immediate notification to the trial's designated representative. For all AEs, it is necessary that sufficient information is obtained by the investigator in order to determine the causality of the AE. Causality is assessed by the investigator and follow-ups may be required until the event resolve or stabilize.

As part of ongoing safety reviews conducted by the Sponsor, any non-serious adverse event that is determined by the Sponsor to be serious will be reported by the Sponsor as an SAE.

To assist in the determination of case seriousness further information may be requested from the investigator to provide clarity and understanding of the event in the context of the clinical trial (Bednar et al., 2015).

8.2 Reporting Period

For SAEs, the active reporting period begins from the time that the subject provides informed consent, which is obtained prior to the subject's participation in the study, ie, prior to undergoing any study-related procedure and/or receiving investigational product, through and including 28 calendar days after the last administration of the investigational product. Should an

investigator be made aware of any SAE occurring any time after the active reporting period, it must be promptly reported. AEs (serious and non-serious) should be recorded on the CRF from the time the subject has taken at least one dose of study treatment through last subject visit (Bednar et al., 2015).

8.3 Definition of an Adverse Event

An AE is defined as any untoward medical occurrence in a clinical investigation subject administered a specific product or medical device when the event does not necessarily have a causal relationship with the treatment or usage. Examples of AEs include but are not limited to (Bednar et al., 2015):

- Abnormal test findings;
- Clinically significant symptoms and signs;
- Changes in physical examination findings;
- Hypersensitivity;
- Progression/worsening of underlying disease;
- Drug abuse;
- Drug dependency.

Additionally, they may include the signs or symptoms resulting from:

- Drug overdose;
- Drug withdrawal;
- Drug misuse;
- Drug interactions;
- Extravasation;
- Exposure during pregnancy;
- Exposure via breast feeding;

 Medication error.

8.4 Abnormal Test Findings

The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

 Test result is associated with accompanying symptoms, and/or

 Test result requires additional diagnostic testing or medical/surgical intervention, and/or

 Test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment, or other therapy, and/or

 Test result is considered to be an AE by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE (Bednar et al., 2015).

8.5 Serious Adverse Events

An SAE is any unexpected medical occurrence that:

◊ Results in death

◊ Is life-threatening

◊ Requires patient hospitalization or prolongation of hospitalization

◊ Results in persistent or significant disability/incapacity

◊ Results in congenital anomaly/birth defect.

An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject or may

require intervention to prevent one of the other AE outcomes, the important medical event should be reported as serious (Bednar et al., 2015).

8.5.1 Potential Cases of Drug-Induced Liver Injury

Abnormal values in aspartate transaminase (AST) and/or alanine transaminase (ALT) concurrent with abnormal elevations in total bilirubin that meet the criteria outlined below in the absence of other causes of liver injury are considered potential cases of drug-induced liver injury and should always be considered important medical events.

Subjects who may present with the abnormal laboratory abnormalities should be further evaluated further in order to determine the exact etiology of the abnormal laboratory values.

For subjects with pre-existing values of total bilirubin above the normal range: Total bilirubin increased by one time the upper limit of normal or 3 times the upper limit of normal.

The subject must return to the investigational site and be evaluated as soon as possible, preferably within 48 hours from awareness of the abnormal results. This evaluation should include laboratory tests, detailed history and physical assessment. In addition to repeating AST and ALT, laboratory tests should include albumin, creatine kinase, total bilirubin, direct and indirect bilirubin, gamma-glutamyl transferase, prothrombin time (PT) and alkaline phosphatase. A detailed history, including relevant information, such as review of ethanol, acetaminophen, recreational drug and supplement consumption, family history, occupational exposure, sexual history, travel history, history of contact with a jaundiced subject, surgery, blood transfusion, history of liver or allergic disease, and work exposure, should be collected.

Further testing for acute hepatitis A, B, or C infection and liver imaging may be warranted (Bednar et al., 2015).

8.6 Hospitalization

AEs reported from studies associated with hospitalization or prolongations of hospitalization are considered serious. In case of any initial admission (even if less than 24 hours) to a healthcare facility meets these criteria.

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical AE is not in itself an SAE. Examples include:

- ◊ Admission because of a preexisting condition not associated with the development of an AE
- ◊ Social admission
- ◊ Administrative admission
- ◊ Protocol-specified admission during a study
- ◊ Optional admission not associated with a precipitating clinical AE
- ◊ Hospitalization for observation without a medical AE

Pre-planned treatments or surgical procedures should be noted in the baseline documentation for the entire protocol and/or for the individual subject (Bednar et al., 2015).

8.7 Severity Assessment

If required on the AE case report forms (CRFs), the investigator may use the categories MILD, MODERATE, or SEVERE to describe the intensity of the AE. For purposes of consistency, these intensity grades are defined as follows: MILD does not interfere with subject's usual function. MODERATE interferes to some extent with subject's usual function. SEVERE interferes significantly with subject's usual function. Note the distinction between the severity and the seriousness of an AE. A severe event is not necessarily also a SAE (Bednar et al., 2015).

8.8 Causality Assessment

The investigator’s assessment of causality must be provided for all AEs (serious and non-serious) and the causal relationship in the CRF, must be recorded as appropriate. An investigator has also to causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an AE; generally the facts (evidence) or arguments to suggest a causal relationship should be provided. If the investigator does not know whether or not investigational product caused the event, then the event will be

handled as “related to investigational product” for reporting purposes, as defined by the Sponsor. If the investigator's causality assessment is "unknown but not related to investigational product", this should be clearly documented on study records. In addition, if the investigator determines an SAE is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with the SAE reporting requirements, if applicable (Bednar et al., 2015).

8.9 Exposure during Pregnancy

If a study subject becomes or is found to be pregnant during the study subject’s treatment with the investigational product, or directly after discontinuing it, the investigator must submit this information, irrespectively of whether an AE has occurred and within 24 hours of awareness of the exposure. The investigator will follow the pregnancy until completion or termination and notify of the outcome. In the case of a live birth, the structural integrity of the neonate can be assessed at the time of birth. In the event of a termination, the reason(s) for termination should be specified. If the outcome of the pregnancy meets the criteria for an SAE (i.e., ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly, the investigator should follow the procedures for reporting SAEs.

Additional information about pregnancy outcomes that are reported as SAEs follows:

- ◊ Spontaneous abortion includes miscarriage and missed abortion;
- ◊ Neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as SAEs.

Additional information regarding the exposure during pregnancy may be requested by the investigator. Further follow-up of birth outcomes will be handled on a case-by-case basis (Bednar et al., 2015).

8.10 Withdrawal Due to Adverse Events

Withdrawal may take place either due to an AE or due to insufficient response and in the case that a subject withdraws due to an SAE, the SAE must be reported in accordance with the reporting requirements defined below (Bednar et al., 2015).

8.11 Eliciting Adverse Event Information

The investigator is responsible for documenting and reporting all directly observed AEs and all AEs spontaneously mentioned by the study subject. In addition, each study subject will be questioned about AEs (Bednar et al., 2015).

8.12 Reporting Requirements

Each AE is to be assessed to determine if it meets the criteria for SAEs. If an SAE occurs, expedited reporting will follow local and international regulations, as appropriate (Bednar et al., 2015).

8.12.1 Serious Adverse Event Reporting Requirements

If an SAE occurs, it must be notified within 24 hours to the investigator. In particular, if the SAE is fatal or life-threatening, notification must be made immediately, irrespective of the extent of available AE information. This timeframe also applies to any additional new information (follow-up) on previously forwarded SAE reports as well as to the initial and follow-up reporting of exposure during pregnancy and exposure via breast feeding cases.

In the case that the investigator does not become aware of the occurrence of an SAE immediately, he has to report it within 24 hours after learning of it and document the time of his/her first awareness of the AE.

In addition, an investigator may be requested to obtain specific additional follow-up information, which, in general, will include a description of the AE in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causes. Information on other possible causes of the event, such as concomitant medications and illnesses

must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible (Bednar et al., 2015).

8.12.2 Non-Serious Adverse Event Reporting Requirements

All AEs will be reported on the AE section of the CRF. AEs should be reported using concise medical terminology on the CRFs as well as on the form for collection of SAE information (Bednar et al., 2015).

CHAPTER 9

INFORMATION ABOUT TREATMENTS ADMINISTERED

9.1 Allocation to Treatment

Investigators will use an Interactive Response System (IRS) to in order to assign a unique subject identification number sequentially to each subject who has signed the Informed Consent Document (ICD). This identifying number is going to be retained throughout the duration of the study participation. Subject eligibility for participation in the active treatment phase of the protocol will be determined following the assessments on Day 1 (Baseline). The investigator will obtain a randomization number and study medication assignments utilizing the IRS. The randomization number will be recorded once in the CRF. The subject will receive the appropriate study medication as assigned by the IRS (Bednar et al., 2015).

9.2 Breaking the Blind

Since the study will be double-blinded, the study drug will be administered to each subject according to the randomization schedule and protocol by a third party, not involved in the study In order to maintain the double-blinded conditions of the study.

The subject and investigator will be blinded to whether the subject receives magnesium aspartate or placebo sodium aspartate during any given period. In order to have an ensured accuracy of the study drug treatment, an unblinded monitor will perform study drug accountability and reconciliation procedures, who is going to remain blinded to all other study data. All other

study personnel will be blinded to the identity of the study drug including a blinded monitor who will remain blinded to treatment until all monitoring for the study has been completed.

To minimize the potential for bias, treatment randomization information will be kept confidential by specific personnel and will not be released to the investigator or investigator site personnel until the study database has been locked.

At the initiation of the study, the study site will be instructed on the method for breaking the blind. Blinding codes should only be broken in emergency situations for reasons of subject safety. In any case, when the blinding code is broken, the reason must be fully documented and entered on the case report form (CRF) (Bednar et al., 2015).

9.3 Drug Supplies

9.3.1 Formulation and Packaging

The magnesium aspartate and the placebo sodium aspartate diluted in lemonade oral solutions will be supplied by the sponsor and presented to the subjects in individual dosing containers at the Clinical Research Unit. The study may additionally utilize a central pharmacy for preparation of magnesium and placebo oral solution for some or all of the study sites. The prednisone oral dosing will also be dispensed at the Clinical Research Unit (Bednar et al., 2015).

9.3.2 Preparation and Dispensing

Magnesium and placebo oral dosing solutions will be prepared in the Clinical Research Unit by 2 operators, one of whom being necessarily a qualified pharmacist. Details of dose preparation and appropriate storage conditions for the solutions will be given in separate Extemporaneous Dispensing Record (EDR). For achieving the ensured accuracy of the study drug treatment, an unblinded monitor will perform study drug accountability and reconciliation procedures (Bednar et al., 2015).

9.3.3 Administration

Investigator site personnel will dispense study medication at visits as specified in the EDR. Dosing should occur from approximately 7 am to 8 pm. Medication errors are reportable irrespective of the presence of an associated AE/SAE, including:

- ◊ Medication errors involving subject exposure to the product.
- ◊ Potential medication errors not foreseen in the protocol.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error must be captured on an adverse event (AE) CRF page. In the event of medication dosing error, the sponsor should be notified immediately (Bednar et al., 2015).

9.3.4 Compliance

All doses of study drug will be prepared by a study pharmacist and administered by study staff. The records of the pharmacy subject chart must document the composition and administration, respectively, of study drug for each subject at each dose. Date and time of administration will be recorded in the subject chart. A second site staff member should confirm documentation of records. This confirmation should be documented in each subject chart (Bednar et al., 2015).

9.4 Drug Storage and Drug Accountability

The investigator, or an approved representative, e.g., pharmacist, must ensure that all investigational product is stored in a secured area under recommended storage conditions and in accordance with applicable regulatory requirements. Storage conditions stated in the Investigator Brochure (IB), Core Data Sheet (CDS), United States Package Insert (USPI), Summary of Product Characteristics (SPC), or Local Product Document (LPD) may be superseded by the label storage. All study drugs will be accounted for via the drug accountability forms. For any deviations from the recommended storage conditions, the sponsor should be notified and the material placed in quarantine until an appropriate assessment can be made of the deviation. At the end of the study, Sponsor will provide instructions as to disposition of any unused investigational product. Investigators and site staff are reminded to check temperatures daily and ensure that thermometers

are working correctly as required for proper storage of investigational products (Bednar et al., 2015).

9.5 Concomitant Medication(s)

All allowed medication should be maintained at a stable dose from the day of the screening and through completion of study treatment. Any change should be carefully reviewed by the investigator and the study Medical Monitor for any potential to impact subject safety or the study efficacy endpoints.

All concomitant medications taken during the study must be recorded with indication, daily dose, and start and stop dates of administration and all subjects are supposed to be questioned about concomitant medication at each clinic visit.

Note: A Prohibited/Allowed Concomitant Medication guidance document has been issued to each site in addition to the specifications below.

The following drugs and classes of drugs are prohibited as concomitant medications after the Screening Visit (Bednar et al., 2015):

- ◊ amphotericin B
- ◊ cyclosporine
- ◊ digoxin, digitalis
- ◊ St. John's wort
- ◊ Systemic steroids (topical steroids allowed)
- ◊ antifungal medication such as itraconazole, ketoconazole, posaconazole, voriconazole
- ◊ birth control pills and other hormones
- ◊ Salicylate, aspirin dosing >925 mg/day, warfarin, coumadin
- ◊ Diuretics, furosemide
- ◊ the hepatitis C medications boceprevir or telaprevir

- ◊ HIV or AIDS medicine such as atazanavir, delavirdine, efavirenz, fosamprenavir, indinavir, nelfinavir, nevirapine, ritonavir, saquinavir;
- ◊ insulin or diabetes medications per mouth
- ◊ a Non-Steroidal Anti-Inflammatory Drug (NSAID) such as aspirin, ibuprofen (Advil, Motrin), naproxen (Aleve), celecoxib, diclofenac, indomethacin, meloxicam, and others
- ◊ seizure medications such as carbamazepine, fosphenytoin, oxcarbazepine, phenobarbital, phenytoin, primidone
- ◊ the tuberculosis medications isoniazid, rifabutin, rifapentine, or rifampin
- ◊ chemotherapeutic drugs only (e.g., cis-platinum, carboplatin, present or previous use)
- ◊ Any other drug or supplement that presents risk for impact on hearing or the study efficacy assessments e.g. Fish Oil -omega-3 polyunsaturated fatty acids, Vitamin B12-cyanocobalamin, Vitamin D3-cholecalciferol
- ◊ Medication interacting with prednisone such as Advair Diskus (fluticasone / salmeterol), Cymbalta (duloxetine), Lipitor (atorvastatin), Lyrica (pregabalin), Nexium (esomeprazole), Plaquenil (hydroxychloroquine), Prilosec (omeprazole), ProAir HFA (albuterol), Singulair (montelukast), Spiriva (tiotropium), Symbicort (budesonide / formoterol), Synthroid (levothyroxine), Tylenol (acetaminophen), Xanax (alprazolam), Zyrtec (cetirizine)
- ◊ Medication interacting with magnesium aspartate such as Tivicay (dolutegravir), Triumeq (abacavir / dolutegravir / lamivudine), abacavir / dolutegravir / lamivudine, dolutegravir.

Medications taken within 28 days before the first dose of study medication will be documented as a prior medication. Medications taken after the first dose of study medication will be documented as concomitant medications (https://www.drugs.com/drug-interactions/prednisone.html; https://www.drugs.com/drug-interactions/magnesium-aspartate-index.html?filter=3&generic_only=).

CHAPTER 10

STATISTICAL CONSIDERATIONS

10.1 Sample size determination

Depending on the type of study design and the studies outcome, there are several different formulas for the sample size calculation. When an RCT is about to be performed, it is a matter of great importance to include enough patients in the study arms of the trial, in order to find as statistically significant a difference that we assume there is in the population (Noordzij et al., 2010).

In practice, there are several complicating factors regarding the sample size determination presenting severe practical difficulties.

The first complication is that the formula is only approximate and based on the assumption that the test of significance will be carried out using a *known* standard deviation. The problem is that we do not know what the sample standard deviation will be until we have run the trial but we need to plan the trial before we can run it. Thus we have to make some sort of guess as to what the true standard deviation is for the purpose of planning, even if for the purpose of analysis this guess is not needed.

Secondly, a further source of uncertainty into sample size calculation is introduced which is not usually taken into account in any formulae commonly employed.

In practice the statistician tries to obtain a reasonable estimate of the likely standard deviation by looking at previous trials.

The third complication is that there is usually no agreed standard for a clinically relevant difference. In practice some compromise is usually reached between 'true' clinical requirements and practical sample size requirements.

Fourth, the levels of significance and of statistical power of the statistical test are themselves arbitrary. Frequently, the values chosen in our example (α=0.05 and 1-β=0.80 or 80%) are the ones employed. The level of significance α is the probability of a type I error, given that the null hypothesis is true, while β is the probability of a type II error, given that the alternative hypothesis is true.

The problem that this raises is frequently ignored. However, where this requirement applies, unless the sample size is increased to take account of it, the power will be reduced (Senn, 2008).

Regarding our study, the simple formula below is adequate for giving a basic impression of the calculations required to establish a sample size:

$$n = \frac{4 \cdot (Zcrit + Zpwr)^2 \cdot \sigma^2}{\Delta^2}$$

Where:

n is the total sample size (the sum of the sizes of both comparison groups),

σ is the assumed SD of each group (assumed to be equal for both groups),

Zcrit is the value of the Normal distribution which cuts off an upper tail probability of **a/2**, for a desired level of significance **α**,

Zpwr is the value of the Normal distribution which cuts off an upper tail probability of **β**, for a desired statistical power **1-β**, and

Δ is the minimum expected difference between the two means.

On the basis of preliminary results from the previous phases of this study, as investigators, we estimate that the magnesium prednisone combination treatment will help the study subjects have an improvement of PTA results by 20 dB, whereas prednisone alone only by 10 dB.

The above equation will be used to determine the sample size for a study with the magnesium and placebo both combined with prednisone, with an expected mean difference of 10 dB and an expected standard deviation of 27.31 dB (Bogaz et al., 2014; Röhrig et al., 2010).

The two-tailed unpaired t-test is to be used, with the level of significance of α=5% and the power of 80%. According to statistical Tables of Normal distribution, $Z_{0.8} = 0.8416$ and $Z_{0.975} = 1.96$. If these values are inserted into the above equation, they give the total sample size as follows (Senn, 2008):

$$n = \frac{4 \cdot (Z_{0.975} + Z_{0.8})^2 \cdot \sigma^2}{\Delta^2} = \frac{4 \times (1.96 + 0.8416)^2 \times 27.31^2}{10^2} = 234$$

Both *Z*crit and *Z*pwr are cutoff points along the x axis of a standard normal probability distribution that demarcate probabilities matching the specified significance level and statistical power, respectively. The two groups that make up ***n*** are assumed to be equal in number, and it is assumed that two-tailed statistical analysis will be used (Eng, 2003).

Assuming a 15% drop-out rate of the initial number of subjects enrolled to this trial, a total number of n=270 subjects will have to be randomized.

10.2 Efficacy Analysis

Primary Efficacy Analysis

All subjects that complete at least the first treatment dose will be included in the efficacy data analyses as appropriate. The primary endpoint for this study is the change from baseline to Tmax in Pure Tone Audiometry (PTA), averaged over 2 kHz and 4 kHz frequencies measured in

the worst ear. Descriptive statistics (arithmetic mean, sample standard deviation, median, minimum and maximum) will be computed and tabulated by treatment.

Significant difference with regards to the primary endpoint mean change between the study groups will be determined using two-sample independent t-test (2-sided) at 5% level of significance (Bednar et al., 2015).

Analysis of Secondary Efficacy Endpoints

The secondary efficacy endpoints are:

- Change from baseline in PTA, averaged over 2 kHz and 4 kHz frequencies.
- Change from baseline in SDS measured in the worst hearing ear as defined by PTA at baseline.
- Change from baseline in SINT.
- Change from baseline in Tinnitus Severity Ranking Scale.

The analysis of the secondary endpoints of change from baseline in PTA, SDS, SINT, Tinnitus Severity Ranking Scale will be conducted using the Hotelling's T square test for two independent samples.

The Speech Discrimination Score (SDS) will be analyzed using the total number of phonemes correctly repeated back by the subject.

Subsequently the mean change from baseline values will be compared between each of the two study groups.

Descriptive statistics (arithmetic mean, sample standard deviation, median, minimum and maximum) will be computed and tabulated by treatment for all secondary endpoints (Bednar et al., 2015).

10.3 Safety Analysis

The Safety Analysis population consists of all randomized subjects who receive at least one dose of randomized study drug. The term "on treatment" refers to the period between the first administration of study drug and two days after the last administration of study drug. This period will be the basis for the safety analysis.

Safety and tolerability will be evaluated taking into consideration the patients' vital signs (supine and standing BP and pulse rate), ECG and clinical laboratory changes from baseline will be assessed for clinically relevant change.

Adverse events, ECG, blood pressure (supine and standing), pulse rate, and safety laboratory data will be reviewed on a regular basis during the study to evaluate the safety of subjects. Any laboratory, ECG and BP abnormalities of potential clinical concern will be described. Safety data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate.

In case of any untoward findings identified on physical and/or neurologic exams conducted after the administration of the first dose of study medication, those findings meet the definition of an adverse event.

Effects of treatment will be evaluated using descriptive statistics (mean, standard deviation, median, minimum and maximum) for baseline, post-baseline and change from baseline values for each treatment. Statistical inference might be made for certain adverse events of medical interest.

Primary Safety Analysis

The principal safety end points is a composite of major and no major clinically relevant adverse events. The principal safety objective of this study is to demonstrate that the combination therapy of magnesium with corticosteroids is superior to corticosteroids alone.

10.4 Interim Analysis

An interim analysis will be performed, when either 50% of the primary efficacy endpoint events, as reported by the Clinical Endpoint Committee (CEC), have occurred, or at a maximum of 36 months after the first subject is randomized. The purpose of this interim analysis is to stop the study early if it is unlikely to establish non-inferiority for the primary efficacy endpoint, if the study were to run to completion. In other words the objective of the interim analysis is to stop the study early due to lack of efficacy. The study will NOT be terminated early to declare non-inferiority.

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